UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF MARYLAND
SOUTHERN DIVISION

INVITROGEN CORPORATION, (FORMERLY LIFE TECHNOLOGIES, INC.) Plaintiff (DJ action Defendant), v. CLONTECH LABORATORIES, INC. Defendant (DJ action Plaintiff).	Civil Action No. AW-96-4080 AW-00-1879

DECLARATION OF JAMES J. CHAMPOUX

I, James J. Champoux, hereby declare and state as follows:

1. I reside at 4101 N.E. 186th St., Seattle WA 98155, and am currently employed by the University of Washington, as a Professor of Microbiology. I have been employed in that capacity for 28 years.

2. I have been retained by the plaintiff, Invitrogen Corporation, in the above-referenced matter to, if called upon, provide expert testimony regarding various issues within my area of expertise, including the issues of protein purification, enzyme assays, protein structure and function, gene engineering, and biochemistry. I have previously provided a report that sets forth those matters upon which I am prepared to testify, if necessary. A copy of my expert report relating to the issues set forth herein is attached (Exhibit 1). I have also attached a copy of my *curriculum vitae* (Exhibit 2), which demonstrates my expertise in the fields of enzymology,

retrovirology and molecular modification of enzyme function, and my knowledge with respect to what a skilled scientist in this field would have known and what techniques were available to such a scientist around 1988.

3. I have reviewed the specifications of the patents at issue, United States Patent Nos. 6,063,608 ("the '608 patent"), 5,668,005 ("the '005 patent"), and 5,244,797 ("the '797 patent") (the "patents-in-suit"), together with information and technology available in January 1988.[1] From this review, I have concluded that the scientific teachings of the patents-in-suit, when considered together with the knowledge of the techniques that were available to a scientist skilled in enzymology and molecular modification of enzyme function in 1988, would have allowed such a scientist, through the practice of only routine experimentation, to substitute an amino acid in the RNase H region identified by the patents-in-suit, to prepare an RNase H-minus reverse transcriptase ("RT"). The factual bases for this conclusion are as follows.

4. The underlying molecular techniques for achieving modifications in gene structure and sequence were routine as of 1988. Such techniques would have included, for example, techniques for introducing deletions, insertions and substitutions (single point mutations or mutations of more than one nucleotide in a sequence) at a precise location in the coding sequence of any isolated gene in order to modify the sequence of an encoded protein, such as an enzyme, thus leading to modification(s) in the enzyme activity. The fact that such techniques were well known is evidenced by the availability of two journal articles published in 1984. One describes the production of point mutations using oligonucleotide-based mutagenesis (Zoller and Smith, *DNA* vol. 3, p. 479-488, attached as Exhibit 3) and the other describes a method for carrying out mutagenesis by inserting linkers at restriction enzyme recognition

sequences (Lobel and Goff, *Proceedings National Academy Sciences U.S.A.* vol. 81, p. 4149-4153, attached as Exhibit 4). In 1987, yet another method for carrying out oligonucleotide-directed site-specific mutagenesis was described in the widely-used serial called Methods in Enzymology (Kunkel, Roberts and Zakour, *Methods Enzymology*, vol. 154, p. 367-382, attached as Exhibit 5). Moreover, other scientific articles published in 1985-1986, Ivanoff *et al.*, *Proc. Natl. Acad. Sci. USA*, vol. 83, p. 5392-5396 (1986) (Exhibit 6) and Amuro *et al.*, *J. Biol.Chem.*, vol. 260, p. 14844-14849 (1985) (Exhibit 7) , demonstrate that it was routine for scientists in this time frame to apply the technique of oligonucleotide-directed mutagenesis to inactivate enzyme function through substitution modifications at the site of highly conserved residues.

5. In 1988, the sequence of the Moloney murine leukemia virus RT gene, the example taught in the '608 specification, was known. Oligonucleotides—short stretches of designed DNA sequences—were also known at the time to be useful for a number of techniques, including site-directed mutagenesis in which a particular nucleotide(s) is targeted for mutation, as exemplified by Zoller and Smith. Thus, a person skilled in the relevant fields of the patents-in-suit would be able to generate point mutations at specific locations in a known RT gene sequence.

6. In the patents-in-suit, Drs. Gerard and Kotewicz proved that the RNase H activity could be reduced or eliminated without affecting the RT polymerase activity by modifying the underlying gene. They did this by analyzing the sequence of the RT protein (see below), modifying it in various ways, and then testing the modified proteins, ultimately showing that the RNase H activity resided between amino acids 503 and 611 of the RT enzyme (Figure 2, '608

[1] I understand that the patents-in-suit each contain the same patent specification. Thus, for the sake of simplicity, any of my specific references will be confined to the '608 specification as exemplifying the patents in general.

Specification).[2] When the gene was modified to remove the RT structure beyond amino acid 611, the resulting modified enzyme retained RT activity and RNase H activity (Figure 2, "pRT603", '608 Specification). However, when the structure was modified to remove the entire region beyond amino acid 503, the RNase H activity was eliminated (Figure 2, "pRTdEcoRV-C", '608 Specification). This observation demonstrated that the 503-611 region was critical to RNase H activity, and that the RNase H activity could be eliminated without appreciably affecting the RT polymerase function.

7. The Kotewicz and Gerard discovery built upon the earlier work of Johnson *et al.*, *Proc. Natl. Acad. Sci., USA, 83*:7648-7652 (1986) (Exhibit 8), which is referenced at columns 2, 16 and 17 of the '608 patent. Using computerized sequence analysis, Johnson *et al.* observed that there are 10 amino acids found at the same position in the sequences of the RNase H activities from various organisms, including Moloney Murine leukemia virus RT, four other retroviral reverse transcriptases, and *E. coli* RNase H. (*See* Figure 2 of Johnson *et al.*). The Kotewicz and Gerard discovery, together with the information contained in Johnson *et al.*, provided the necessary roadmap for scientists to apply standard molecular modification techniques, such as amino acid deletions, insertions and substitutions, to knock out RNase H function from RT by any of a number of approaches.

8. One of ordinary skill in the art in 1988 would have known that amino acid sequences that are conserved in various organisms signify their importance to that particular protein's activity. An ordinarily skilled artisan would have also known that these conserved amino acid sequences are conserved because they important to the function of the protein, for

[2] It has more recently been determined that due to the presence of added amino acids from the cloning vector, the precise location of the region identified by Kotewicz and Gerard within the natural RT gene begins after amino acid 498 rather than 503. Since this shift does not affect the conclusions herein, the original designations given in the '608 specification, 503-611, will be used in the remainder of this declaration.

example, that they may serve a role in the catalytic activity of the protein. Given the information provided by Kotewicz and Gerard discussed above, such a worker would have known that substituting highly conserved amino acids with dissimilar amino acids in the region defined by Kotewicz and Gerard, might inactivate the enzymatic activity of the molecule. Combining the teachings of Johnson, *et al.*, which identified the specifically conserved amino acids, with the teachings of the '797, '005, and '608 patents, which taught that the RNase H activity of retroviral reverse transcriptases is confined to the 503 to 611 region of the molecule, one of ordinary skill in the art in 1988 would have focused his or her efforts on the seven conserved amino acids between amino acid residues 503 and 611 of RT. It would have been routine experimentation for one of ordinary skill in the art to simply substitute one or more of those conserved amino acids for dissimilar amino acids, and determine whether the RNase H activity was knocked out.

9. Drs. Kotewicz and Gerard published their discovery concerning the importance of the 503 to 611 region of RT in the January 1988 edition of the well-known scientific journal, *Nucleic Acids Research* (Exhibit 9). Through their publication, Kotewicz and Gerard provided scientists with the necessary information to design, construct and test modified RTs having reduced or eliminated RNase H. This view is supported by the fact that other scientists, publishing shortly after Kotewicz and Gerard's publication, confirmed the applicability of their approach by designing and producing various versions of RNase H-minus RT. Tanese and Goff at Columbia University published an article in the March 1988 issue of the *Proceedings of the National Academy of Sciences, USA*, confirming the Kotewicz and Gerard discovery by showing that insertion mutations in the same sensitive region of the RT coding sequence eliminated the RNase H function. (Exhibit 10, Figure 2B, page 1778). Importantly, Tanese and Goff knocked

out the RNase H activity using insertions rather than deletions like Kotewicz and Gerard, which nevertheless achieved essentially the same result.

10. The work of Kotewicz and Gerard was further confirmed by the *Journal of Virology* article of Repaske *et al.* ("Repaske", Exhibit 11), submitted only six months after the Kotewicz and Gerard publication. Repaske confirms the broad enablement of Kotewicz and Gerard by making substitution mutations that knock out RNase H activity in the 503-611 region of the M-MLV RT gene.

11. Thus, in a matter of just a few months after Kotewicz and Gerard published their paper and filed their patent application, two different groups (Tanese and Goff, and Repaske *et al.*) published or submitted papers confirming the broad enablement of the patents-in-suit—that the RNase H activity could be selectively knocked out while retaining RT polymerase activity—using either of two well-known gene modification techniques (insertion or substitution mutations) and each introducing their modifications in the very region of RT, between amino acids 503 and 611, first taught by Kotewicz and Gerard.

12. Thus, it is evident that one of ordinary skill would have had to practice only routine experimentation to use the information provided by Kotewicz and Gerard in their patent application to arrive at a substitution modification (*i.e.*, point mutation) that resulted in reduced RNase H function of the RT. Such an individual would have been apprised of the 503-611 region identified by Kotewicz and Gerard, as well as the 10 conserved amino acids identified by Johnson *et al.* The individual would have then combined these two pieces of information and found that 7 of the 10 conserved amino acids of Johnson *et al.* are found in the region identified by Kotewicz and Gerard. The individual would then have systematically substituted each of these 7 amino acids, for example, with amino acids that have a much different charge property,

and tested the effect of the substitution using the screening methodology described in, for example, column 13 of the '608 patent ("Enzymes Assays"). While such a screening procedure may well have taken a fair amount of time, such experimentation is quite routine in nature as further evidenced by Ivanoff *et al.* and Amuro *et al.* In fact, we know from Repaske *et al.* that this screening technique would have been successful, since Repaske *et al.* confirmed that two of those 7 amino acids are indeed essential for RNase H activity.

I declare under penalty of perjury that, to the best of my knowledge, the foregoing is true and correct.

Date: 3/7/01

By: James J. Champoux

James J. Champoux